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Gymnasterol, a new antitumor steroid against IGF-dependent cells from *Gymnascella dankaliensis*

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Abstract—A new antitumor substance, gymnasterol (**1**), was isolated from the culture broth of *Gymnascella dankaliensis*. The structure of **1** was determined to be a novel ergostane steroid on the basis of NMR studies. Gymnasterol selectively inhibited IGF-1-dependent growth of MCF-7 human breast cancer cells.

Insulin-like growth factors (IGFs) play a key role in human cancer progression. IGF signals through IGF-1 receptor are known to be significant for tumor cell growth and survival.[1] Thus, selective inhibitors of IGF signal transduction are expected to be new anticancer agents against IGF-dependent tumor cells. In the course of our screening for inhibitors of IGF-dependent cell growth or survival, a fungal strain identified as *Gymnascella dankaliensis* was found to produce a new active substance, gymnasterol (**1**, Fig. 1).

The producing organism was cultivated in flasks containing a medium consisting of 5.0% glucose, 1.0% soybean meal, 0.4% meat extract, 0.4% Polypepton, 0.1% yeast extract, 0.25% sodium chloride and 0.5% calcium carbonate (pH 7.0) on a rotary shaker at 25°C for 4 days. The acetone extract of the whole broth (2 l) was evaporated to an aqueous concentrate and then partitioned between ethyl acetate and water. The organic layer was subjected to silica gel column chromatography with chloroform–methanol (25:1). The active fraction was purified by HPLC using a Senshu Pak PEGASIL ODS column with 80% acetonitrile to give a colorless oil of **1** (13.5 mg).[2]

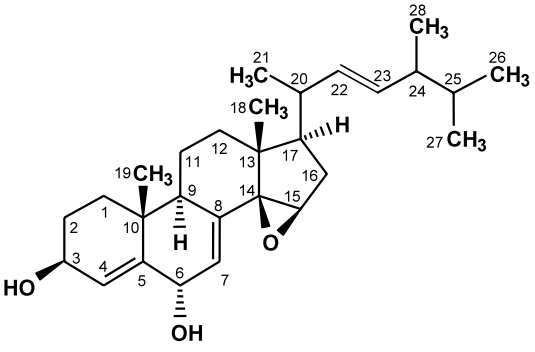

Figure 1. Structure of gymnasterol (**1**).

The molecular formula of **1** was determined to be $C_{28}H_{42}O_3$ from high-resolution FAB-MS (m/z 426.3127, M^+, –0.7 mmu error). The ^{13}C NMR spectrum of **1** confirmed the presence of 28 carbons and an HMQC experiment established all one-bond ^1H–^{13}C connectivities.[2] A COSY experiment identified two separate proton spin systems as shown in Figure 2. The allylic couplings were confirmed by ^1H–^{13}C long-range correlations from 4-H to C-6, from 7-H to C-5 and C-9, and from 11-H to C-8 in the HMBC spectrum. Long-range couplings from 18-H_3 to C-12, C-13, C-14 and C-17 joined the two partial structures via C-13. A tetracyclic carbon skeleton was constructed by long-range correlations from 19-H_3 to C-1, C-5, C-9 and C-10, from 7-H to C-14, from 15-H to C-8, and from 16-H to C-14 (Fig. 2). The existence of an epoxide ring at C-14 and C-15 was required by their ^1H and ^{13}C chemical shifts and the molecular formula. The geometrical configuration of C-22 was established to be *E* based on a large vicinal coupling constant ($J_{22-23}=15.0$ Hz). From these results, the planar structure of **1** was determined as shown in Figure 2.

Keywords: gymnasterol; insulin-like growth factor; steroid; antitumor substance.

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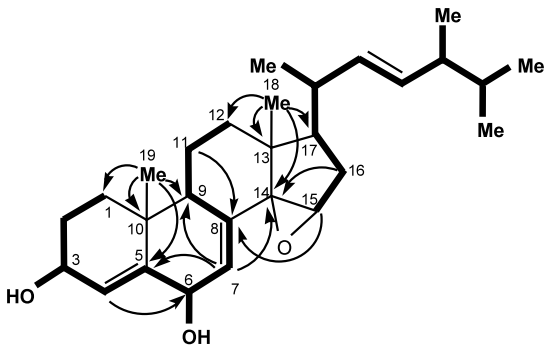

Figure 2. Planar structure of **1** derived from COSY (bold lines) and HMBC (arrows).

The relative stereochemistry of **1** was analyzed by a NOESY experiment. NOEs on 9-H, 18-H_3 and 19-H_3 as shown in Figure 3 revealed typical steroidal configurations for C-9, C-10 and C-13. The two hydroxyl groups were assigned to 3β and 6α based on NOEs between 1-Hα and 3-H, and between 19-H_3 and 6-H. NOEs from 15-H to 7-H and 9-H indicated that a *cis* epoxide existed in a β arrangement. The side chain at C-17 was arranged in a β orientation by NOEs from 18-H_3 to 20-H and 21-H_3. Thus the structure of **1** was established as shown in Figure 1. Structurally related 14,15-epoxyergostane steroids are rarely found in natural products, which include gymnasterone B, a cytotoxic metabolite from *G. dankaliensis*.[3] Further stereochemical studies are now underway.

Gymnasterol (**1**) inhibited the growth of MCF-7 human breast cancer cells[4] in a serum-free medium containing IGF-1 (30 ng/ml) with an IC_{50} of 48 ng/ml. In the presence of 0.5% fetal bovine serum, **1** exhibited weak cytotoxic activity against MCF-7 cells (IC_{50} 2.4 μg/ml), although serum pretreatment did not reduce the activity of **1**. Such selective activity of **1** was not observed in Colo320DM human colon cancer, MDA-MB-231 human breast cancer, HeLa human cervical cancer, MKN-45 human gastric cancer, Saos-2 human osteosarcoma or 3Y1 rat fibroblast cells (IC_{50} 1.2∼6.2 μg/ml). A variety of unique activities have been reported for steroidal compounds, which include Na^+/K^+ ATPase inhibitors,[5] an angiogenesis inhibitor[6] and an interleukin-6 receptor antagonist[7] as well as steroid hormone agonists or antagonists. The unique structural features of gymnasterol combined with its interesting activity make it a possible candidate for anticancer therapeutics and expand the area of steroid chemistry.

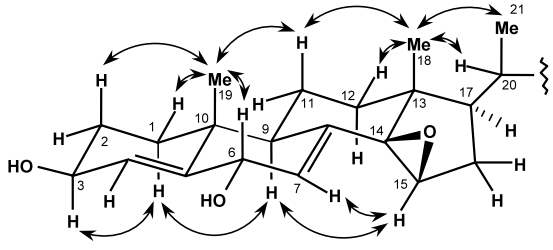

Figure 3. NOESY analysis for the fused-ring part of **1**.

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2. Data for gymnasterol (**1**): $[\alpha]_D^{25}$ −22° (*c* 0.20, MeOH); IR (KBr) ν_{max} 3390, 2960, 2870, 1660, 1450, 1370, 1050, 980, 870 cm^{-1}; UV λ_{max} (ε) 268 nm (2,100) in MeOH; ^{13}C NMR δ 146.2 (C-5), 136.0 (C-8), 135.5 (C-22), 132.9 (C-23), 124.1 (C-7), 121.9 (C-4), 72.7 (C-14), 67.9 (C-15), 66.5 (C-6), 65.6 (C-3), 53.6 (C-17), 48.2 (C-9), 45.3 (C-13), 44.0 (C-24), 40.1 (C-12), 39.0 (C-20), 38.2 (C-10), 33.9 (C-25), 32.6 (C-1), 29.6 (C-2, C-16), 23.4 (C-21), 22.3 (C-19), 21.3 (C-11), 20.4 (C-27), 20.1 (C-26), 18.1 (C-28), 15.9 (C-18) in acetone-d_6; ^1H NMR δ_H (multiplicity, *J*= Hz) 5.80 (dd, 3.0, 2.0, 4-H), 5.58 (t, 2.0, 7-H), 5.39 (dd, 15.0, 8.0, 22-H), 5.15 (dd, 15.0, 8.0, 23-H), 4.64 (m, 6-H), 4.05 (m, 3-H), 3.73 (d, 7.0, 6-OH), 3.55 (d, 6.0, 3-OH), 2.95 (s, 15-H), 2.31 (m, 20-H), 2.14 (m, 9-H), 1.93 (m, 16-H_2), 1.89 (m, 24-H), 1.75 (m, 1-Hβ), 1.74 (m, 2-Hβ), 1.68 (m, 12-Hβ), 1.63 (m, 17-H), 1.61 (m, 11-Hα), 1.56 (m, 1-Hα), 1.54 (m, 11-Hβ, 12-Hα), 1.47 (m, 2-Hα, 25-H), 1.05 (s, 19-H_3), 1.04 (s, 18-H_3), 0.96 (d, 7.0, 28-H_3), 0.91 (d, 7.0, 21-H_3), 0.86 (d, 7.0, 27-H_3), 0.84 (d, 7.0, 26-H_3) in acetone-d_6.
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